Enhanced Potency of Anticancer Drug, Bieomycin by ELF Magnetic Fields

Enhanced Potency of Anticancer Drug, Bleomycin by ELF Magnetic Fields

M. Kakikawa, T. Fujihata, M. Iwahara and S. Yamada
Kanazawa University

The anticancer drug that spreads in the whole body acts on not only the cancer cells but also normal cells and it causes serious side effects. If it's possible that magnetic fields enhance the potency of anticancer drug on target region only, the dosage can be reduced and thus side effects can be suppressed. In this study, we investigated effects of 60 Hz magnetic fields on anticancer drug, bleomycin potency. As a result of the experiment using plasmid DNA, DNA strand break action of bleomycin was enhanced by magnetic fields. The viability of the cells incubated with the combination of bleomycin and magnetic fields, were decreased about 40% than that bleomycin only. These result suggested that magnetic fields (60 Hz, 50 mT) caused greater enhancement of the potency of bleomycin.

Key Words: Extremely-Low-Frequency magnetic fields, anticancer drug, bleomycin

1. Introduction

The medicine administered to the inside of the body extends to not only the diseased cells part but also the whole body. The medicine that spreads in the whole body acts on not only the diseased cells but also normal cells. Since the anticancer drugs have strong effects especially, those cause serious side effects. Additionally, cancer cell resistance to many unrelated anticancer drugs is a major obstacle during cancer chemotherapy.

In resent years, there are a few reports about combination effect of magnetic fields and antcancer drug [1-4]. We also found that the potency of anticancer drug, mitomycin C was enhanced by ELF magnetic fields [5].

If it's possible that magnetic fields enhance the potency of anticancer drug on target region only, the dosage can be reduced and thus side effects can be suppressed. Anticancer drug, bleomycin possesses anticancer and antimicrobial properties by DNA strand breakage that depends on the metal-ion (Fe^{2+}, Zn^{2+} ion etc.) and oxygen. Clinically, bleomycin is used in combination chemotherapy with a number of other agents, against several types of notably squamous cell carcinomas and malignant lymphomas [6-8].

In this study, we investigated effects of 60 Hz magnetic fields on anticancer drug, bleomycin potency.

Correspondence: M. Kakikawa, Institute of Nature and Environmental Technology, Kanazawa University, 920-1192, Japan
email: kakikawa@magstar.ec.t.kanazawa-u.ac.jp

2. Materials and Methods

2.1 Magnetic Fields Generator

Extremely-low-frequency magnetic fields generator used in this study was shown in Fig. 1. By exciting the one direction coil of orthogonal oriented, the magnetic fields (60 Hz, up to 50 mT) were generated and uniformed of 1% in experimental area. To avoid the influence of the heat generated by the coil, the temperature change in the experiment area was controlled at 37 °C by circulating the constant temperature water.

In non-exposure as control, the leakage field from the exposure coils was less than 20 μT.

2.2 Bacteria, Plasmid and Bleomycin

Escherichia coli bacteria were used as experimental cells in this study, because *E.coli* cells can be capable of the earliest proliferation in the cells that acted upon by anticancer drug, bleomycin

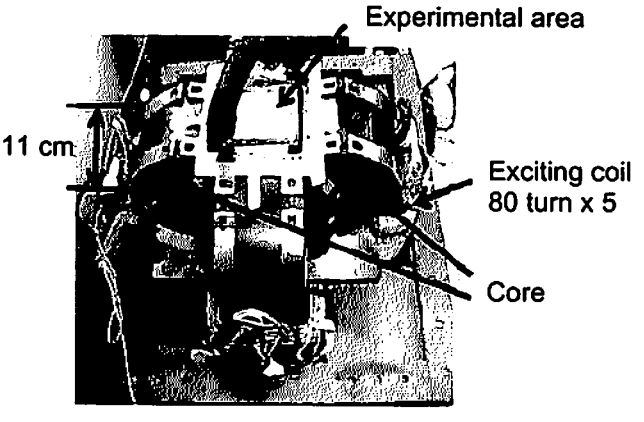

Fig. 1 ELF Magnetic fields generator

as well as human cells. The generation time of *E. coli* bacteria is about 30 minutes, and that time of human cell line is about one day.

The *Escherichia coli* W3110 and XL1-Blue are from National BioResource Project (NIG, Japan). The cells were grown in LB broth (1 % tryptone, 0.5 % Yeast Extract, 1% NaCl, pH7.0) at 37 °C with shaking.

The *E.coli* plasmid pUC119 DNA was from our laboratory stock.

Anticancer drug, Bleomycin was purchased from Nihonkayaku (Tokyo).

2.3 Analysis of Effect of Magnetic Fields on Bleomycin-Iinduced Breakage of Plasmid DNA

The Plasmids DNA exists as super coiled DNA form in *E.coli* and induces open circular DNA form by DNA strand breakage. The effect of magnetic fields on bleomycin-induced breakage of plasmid DNA was evaluated by the following method. The *E.coli* containing plasmid pUC119 (Amp^r) was grown in LB broth containing ampicilin 60 μg/ml at 37 °C with shaking. When the Absorbance-660nm of *E.coli* culture reached about 0.6, late logarithmic growth phase, bleomycin was added to the *E.coli* cells culture with or without the magnetic fields. Then the plasmid pUC119 DNA was extracted from host *E.coli* XL1-Blue and electrophoretically separated on 1 % agarose gels in TAE buffer by a method of J. Sambrook and D.W. Russell [9]. The order of migration of DNA (faster to slower) under these conditions was super coiled DNA and single-strand broken (relaxed), open circular DNA.

DNA breakage action of bleomycin was measured by abundance ratio of open circular (single-strand broken) and super coiled plasmid DNA using image analysis software, WinROOF (Mitani co.), since the plasmids DNA were stained by ethidium bromide and detected with fluorescence on a UV light plate.

3.2 Analysis of Effect of Magnetic Fields on Bleomycin –treated Cell Viability

Influence of magnetic fields on the cell proliferative inhibitory action of the bleomycin was evaluated by the colony assay of *E. coli* bacteria.

E.coli containing no plasmid was grown in LB broth at 37 °C with shaking. When the Absorbance-660 nm of *E.coli* culture reached about 0.3, logarithmic growth phase, bleomycin was added to *E.coli* cells culture with or without the magnetic fields. After treatment of anticancer drug, *E.coli* cells were plated on LB solid agar medium and incubation at 37 °C.

By overnight incubation, colonies of *E.coli* were formed on the solid medium. Because one colony was formed from single viable bacterial cell, the effect of magnetic fields on the viability of bleomycin - treated cells was evaluated by *E.coli* colony count assay.

3. Results

3.2 Effect of Magnetic Fields on Bleomycin- induced Breakage of Plasmid DNA

In previous study, we found that magnetic fields exposure (60 Hz, 50 mT, 5 hours) have effect of enhancing DNA damage action of anticancer drug, mitomycin C [5].

To test whether the magnetic fields enhance the DNA breakage action of anticancer drug, bleomycin, interacting with Fe^{2+} ion, *E.coli* containing plasmid DNA was incubated for 1 to 5 hour in 50 mT magnetic fields and bleomycin or bleomycin only in this study.

Fig. 2 shows that the electrophoresis pattern of plasmids DNA treated with each concentration of bleomycin for 1.5 hour with or without 60 Hz, 50 mT magnetic fields. The plasmids DNA exist as super coiled form in *E.coli* cells (lane 1). As shown in Fig. 2, lane 6 of a positive control, the plasmids DNA were cut and converted from super coiled DNA form to open circular DNA form by bleomycin. By co-exposure to magnetic fields and bleomycin, the amount of open circular DNA was increased about twice than that by bleomycin only (lane 5). This result suggested that DNA break action of bleomycin was enhanced by magnetic fields.

3.2 Effect of Magnetic Fields on Bleomycin– treated Cell Viability

The Effects of magnetic fields on the cell proliferative inhibitive action of bleomycin was evaluated by the viability of *E. coli* bacteria incubated for 1 hour under 60 Hz, 50 mT magnetic fields and bleomycin or bleomycin only. The viability of *E.coli* cells can be measured by colony assay, because a colony is formed by growth of single cell on solid agar medium during overnight incubation.

As a result, the viability of the cells incubated with magnetic fields and bleomycin, was decreased about 40% than that of bleomycin only (Fig. 3).

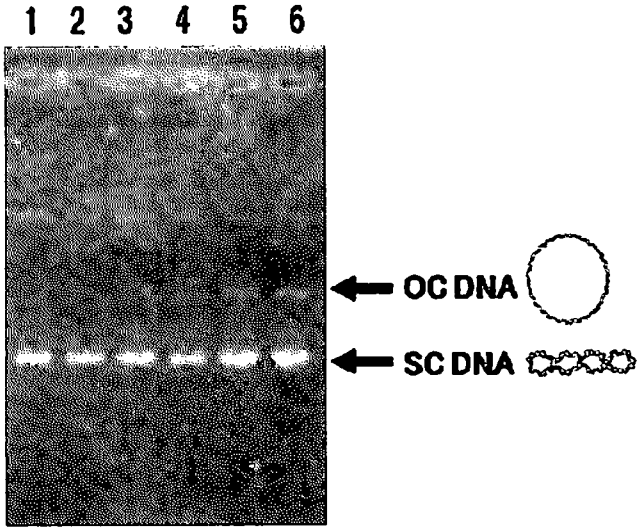

Fig. 2 Effect of magnetic fields on bleomycin- induced breakage of plasmid DNA. Lane 1, bleomycin (0 μM); lane 2, bleomycin (5 μM); lane 3, bleomycin (5 μM) and magnetic fields; lane 4, bleomycin (10 μM); lane 5, bleomycin (10 μM) and magnetic fields; lane 6, bleomycin (25 μM); OC DNA, Open circular plasmids DNA; SC DNA, Super Coiled plasmids DNA

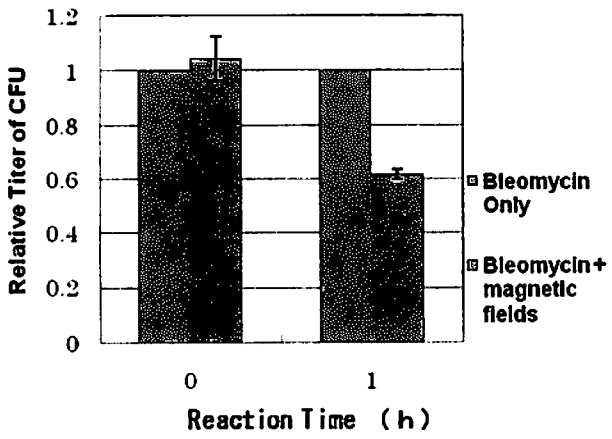

Fig. 3 Effect of magnetic fields on bleomycin –treated cell viability. CFU; colony forming unit

This result suggested that magnetic fields (60 Hz, 50 mT) caused greater enhancement of the potency on the cell proliferative inhibitory action of bleomycin.

4. Discussion

It was found that ELF magnetic fields (60 Hz, 50 mT) enhance the DNA damage action of anticancer drug, mitomycin C by previous study of our group.

The action of bleomycin could receive the more effects of ELF magnetic fields than that of mitomycin C, because bleomycin widely used in chemotherapy, depends on metal-ion (Fe^{2+}, Zn^{2+} ion etc.). To test the above hypothesis, the effect of combination of magnetic fields and bleomycin was estimated on *E.coli* simple cells in this study. These results of DNA breakage and cell viability suggested that the 60 Hz, 50 mT magnetic fields enhance the potency of bleomycin. However, the action of bleomycin could not receive the effects of ELF magnetic fields than that of mitomycin C.

Other group studies indicated that the pulsed magnetic fields have enhancing effect on the potency of anticancer drug, daunorubicin [1], mitomycin [2 and 3], cisplatin [3]. S. Tofani *et al.* reported that the survival time of the C57BL/6 mouse bearing lung carcinoma, treated with cisplatin and exposed to ELF magnetic fields was significantly longer than that of mice treated only with cisplatin or only with pulsed magnetic fields. On the other hand, cyclophosphamide anti-tumor activity was not influenced by ELF magnetic fields in S. Tofani *et al.* study [4]. That is, magnetic fields have neither positive nor negative effects on the action of cyclophosphamide in the mice. These reported indicated that the combination of magnetic fields and chemotherapeutics has positive effect, no negative effect. The role of magnetic fields exposure parameters (frequency, intensity, time, etc.) in antitumor efficacy with antitumor drug have to be studied in greater detail.

It's also not clear that the mechanisms of magnetic fields action on the potency of these antitumor drugs.

How magnetic fields can effect on the action of bleomycin? Bleomycin is thought to exert its biological effects through metal-dependent oxidative cleavage of DNA and RNA in the presence of oxygen [7]. Which stage of an action of bleomycin (on the drug intake, interaction to metal-ion, oxidative DNA breakage, or DNA repair system) does a magnetic field affect? Magnetic fields seem to enhance active oxygen production [4]. When the active oxygen production occurs at the cell membranes, the permeability changed, influencing the cell drug intake [10]. Magnetic fields might effect on the drug intake and oxidative DNA breakage. To test whether magnetic fields affect on the interaction of metal–ion, influencing metal-dependent drug, the study on magnetic fields effects on other anticancer drugs, possessing metal-

nondependent oxidative DNA breakage property, has to investigate.

Since there are many *E.coli* mutant of DNA repair system, it would become clear that magnetic fields affect or no affect on DNA repair system following DNA damage in the cells by using *E.coli* DNA repair mutant cells.

Future studies will attempt to evaluate enhancement of antitumor potency on the human culture cells by magnetic fields and to clear the mechanism of effects on anticancer drug potency by magnetic fields.

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